REMARKS

This Amendment is in response to the Examiner's Office Action mailed September 5, 1995 (Paper No. 6). Claims 1-12 were reviewed by the Examiner. Applicants cancel original claims 1-12 and submit new claims 13-38. Now pending are claims 13-38.

Reconsideration of the application is respectfully requested in view of the above amendments to the claims and the following remarks. For the Examiner's convenience and reference, Applicants' remarks are presented in the order in which the corresponding issues were raised in the Office Action.

I. New Claims 13-38

Applicants currently cancel original claims 1-12 and submit new claims 13-38. Original claims 1-12 were directed to a "system" for real time monitoring of a nucleic acid amplification reaction. In the Office Action, the Examiner indicates that these claims were treated as apparatus type claims. New claims 13-38 are submitted to pursue both apparatus claims and method claims. More specifically, new claims 13-22 are directed to an apparatus for monitoring the formation of a nucleic acid amplification reaction product in real time. Meanwhile, new claims 23-38 are directed to a method for monitoring the formation of a nucleic acid amplification reaction product in real time.

II. Objection To Disclosure

The Examiner objects to the disclosure on the grounds that claim 8 does not end in a period. Claim 8 is presently cancelled. The Examiner also objects to the disclosure on the grounds that the Specification at page 13 does not specify the SEQ. I.D. NOs for the nucleic acid sequences specified. Page 13 has been amended to include SEQ. I.D. NOs for the nucleic acid sequences specified.

III. Rejection Of Claims 1-5 And 7-8 Under 35 U.S.C. § 103

The Examiner rejects claims 1-5 and 7-8 under 35 U.S.C. § 103 as being unpatentable for obviousness over Burg, et al. or Higuchi, et al. (1993), in view of either Gershoni, et al. or Krause, et al.

As the Examiner acknowledges, neither Burg, et al. nor Higuchi, et al. (1993) teach the use of a second fluorescent indicator which produces a signal proportional to the volume of the reaction mixture. The Examiner cites Gershoni, et al. and Krause, et al. as teaching the use of an internal standard.

Applicants traverse the Examiner's combination of Burg, et al. or Higuchi, et al. (1993) with Gershoni, et al. or Krause, et al. on the grounds that it is improper to combine Burg, et al. or Higuchi, et al. (1993) with Gershoni, et al. or Krause, et al. since one of ordinary skill would not consider Gershoni, et al. or Krause, et al. prior art relative to the present invention. Applicants also traverse the Examiner's rejection on the grounds that the cited references fail to teach each and every limitation specified in the claims as amended. On these bases, the Examiner has failed to set forth a prima facie case for obviousness. Applicants therefore respectfully request that the Examiner withdraw the present rejection for obviousness.

A. Summary Of The Independent Claims

Independent claims 1, 24, 36 and 38 specify an apparatus and method for monitoring the formation of a nucleic acid amplification reaction product in real time in which the sample includes a first fluorescent indicator and a second fluorescent indicator.

The first fluorescent indicator produces a first fluorescent signal when illuminated by an excitation beam whose intensity is proportional to the concentration of amplification reaction product in the sample and the volume of the sample illuminated by the excitation beam. Meanwhile, the second fluorescent indicator is homogeneously distributed throughout the sample and produces a second fluorescent signal when illuminated by an excitation beam whose intensity is proportional to the volume of the sample illuminated by the excitation beam.

According to the apparatus and method claims, an excitation beam is transmitted into the sample which illuminates a volume of the sample. In response, the first and second fluorescent indicators produce first and second signals which are measured in order to determine the amount of amplification reaction product present.

B. Neither Gershoni, et al. Nor Krause, et al. Is Prior Art

Applicants traverse the Examiner's rejection for obviousness on the grounds that neither Gershoni, et al. nor Krause, et al. is within the scope of what may be considered as "prior art" relative to the present invention.

Two criteria have evolved for determining whether a reference may be considered prior art relative to an invention: (1) whether the art is from the same field of endeavor, regardless of the problem addressed, and (2) if the reference is not within the field of the inventor's endeavor, whether the reference still is reasonably pertinent to the particular problem with which the inventor is involved. See In re Clay 23 USPQ2d 1058 (1992).

Both Gershoni, et al. and Krause, et al. relate to methods for quantifying chlorophyll fluorescence in photosynthetic cells at very low temperatures (77 °K, i.e., -196 °C). By contrast, the present invention relates to a method and apparatus for the real time detection of amplified nucleic acid sequences. The method and apparatus operate at a temperature range between about 50°C and 95°C. Hence, the present invention involves operating temperatures which are at least 250 degrees above the temperature used in Gershoni, et al. and Krause, et al. In view of the fact that neither Gershoni, et al. nor Krause, et al. relates to the detection and quantification of nucleic acids and since there is at least a 250 degree temperature difference between the assays, one of ordinary skill would not consider Gershoni, et al. or Krause, et al. to be in the same field of endeavor as the claimed invention.

Gershoni, et al. and Krause, et al. also are not reasonably pertinent to the particular problem with which the inventor is involved. As described above, the present invention relates to a method and apparatus for the real time detection of amplified nucleic acid sequences which operate at a temperature at least 250 degrees above the temperature used by Gershoni, et al. and Krause, et al. In view of the significant temperature difference, and the fact that neither Gershoni, et al. nor Krause, et al. is concerned with the automated detection of nucleic acids, these references would not be considered pertinent to the particular problem being addressed by the present invention.

Gershoni, et al. and Krause, et al. also are not reasonably pertinent because these references do not use an internal standard in the manner in which it is used in the present invention. As specified in the independent claims, the second fluorescent signal has an intensity which is proportional to the volume of the sample illuminated by the excitation

beam. By contrast, Gershoni, et al. and Krause, et al. use the internal standard in order to distinguish between normalized spectra. For example, Gershoni, et al. teaches normalizing the spectra by varying the monochromator slit so that each spectrum has an equal emission intensity for the major peak. Gershoni, et al., page 317, Col. 1. As a result of normalizing the spectra, it is not possible compare the relative chlorophyll content in each sample. Gershoni, et al., page 318, Col. 1. An internal standard is therefore used in order to recognize differences in the chlorophyll concentration of different samples. Gershoni, et al., page 318, Col. 1.

Since Gershoni, et al. and Krause, et al. are not from the same field of endeavor as the present invention or reasonably pertinent to the particular problem addressed by the present invention, Applicants submit that Gershoni, et al. and Krause, et al. are not prior art relative to the present invention and cannot be reasonably relied upon in support of the present rejection for obviousness. The Examiner is therefore respectfully requested to withdraw the present rejection for obviousness.

B. <u>The Cited References Do Not Teach Or Suggest All Of The Claim Limitations</u>

In order for a claim to be rendered obvious by one or more references, each and every claim limitation must be taught by the reference(s). Since the Examiner has not cited references which combined teach or suggest the invention as claimed, the Examiner's present rejection for obviousness should be withdrawn.

Independent claim 13 specifies an apparatus for monitoring the formation of a nucleic acid amplification reaction product in real time which includes a fiber optic cable and a lens co-axially disposed with the fiber optic cable for collecting from the sample and transmitting to the fiber optic cable a first fluorescent signal corresponding to the amplified nucleic acid and a second fluorescent signal which serves as an internal standard. As the Examiner acknowledges in the Office Action, neither Burg, et al. nor Higuchi, et al. teach or suggest the use of an internal standard. Hence, neither reference teaches or suggests an optical assembly for collecting to separate signals. As discussed above, Gershoni, et al. and Krause, et al. are not concerned with the real time detection of nucleic acids and operate at a very low temperature. Hence, neither of these references teach or suggest

modifying the optical assembly of Burg, et al. or Higuchi, et al. to collect more than one fluorescent signal.

Independent claims 24, 36 and 38 specify a method for monitoring the formation of a nucleic acid amplification reaction product in real time using a first fluorescent signal which corresponds to the amplified nucleic acid and a second fluorescent signal which serves as an internal standard for determining the volume of the sample illuminated by the excitation beam. Neither Burg, et al. nor Higuchi, et al. teach or suggest the use of an internal standard. Meanwhile, Gershoni, et al. and Krause, et al. do not use an internal standard in order to determine the volume of the sample illuminated. Instead, these references use the internal standard in order to distinguish between normalized spectra. Gershoni, et al., page 318, Col. 1.

Dependent claim 14 specifies a detection and analysis mechanism which provides a readout including a ratio between the intensity of the first fluorescent signal and the intensity of the second fluorescent signal. Since neither Burg, et al. nor Higuchi, et al. teach or suggest the use of an internal standard, these references do not teach the detection and analysis mechanism. Since Gershoni, et al. and Krause, et al. are not concerned with an automated process, these references do not teach or suggest an apparatus having a detection and analysis mechanism as specified. Accordingly, claim 14 is not rendered obvious by the cited references.

Claim 21 specifies a mechanism for heating an optical interface between the lens and the sample to prevent condensation of the sample on the optical interface. Claim 28 specifies a method which includes the step of heating the optical interface to prevent condensation of the sample on the optical interface. Claim 22 depends from claim 21 and claims 29 and 32 depend from claim 28. None of the references cited teach or suggest a mechanism for heating an optical interface to prevent condensation or a method including the step of heating the optical interface to prevent condensation. Accordingly, these claims are not rendered obvious by the cited references.

Claim 23 specifies an apparatus in which the sample holder includes a removable reaction chamber for holding the sample where one wall of the reaction chamber serves as an optical interface which is separated from the sample by an air gap. Burg, et al. teaches moving an optical fiber beneath an array of cuvettes. Hence, Burg, et al. does not teach a

removable reaction chamber where one wall of the reaction chamber serves as an optical interface which is separated from the sample by an air gap. Meanwhile, Higuchi, et al. (1993) teaches the use of a plurality of fiber optic cables where the probe end of each fiber optic cable is fixedly attached to the open top of a PCR tube. Higuchi, et al. (1993), page 4, Col. 1, first paragraph and Col. 2, last paragraph. Hence, Higuchi, et al. (1993) does not teach a removable reaction chamber. There is no teaching or suggestion in the references cited to produce a removable reaction chamber for holding the sample where one wall of the reaction chamber serves as an optical interface which is separated from the sample by an air gap. Claim 23 therefore is not rendered obvious by the cited references.

Claim 34 specifies a method in which the first and second fluorescent indicators are covalently attached to an oligonucleotide having a nucleotide sequence complementary to a portion of a strand of the amplification reaction product, the second fluorescent indicator quenching the fluorescence of the first fluorescent indicator. Attachment of first and second fluorescent indicators to an oligonucleotide is neither taught nor suggested by the references cited by the Examiner in support of the present rejection for obviousness. Claim 34 therefore is not rendered obvious by the cited references.

IV. Rejection Of Claims 1-5 And 7-8 Under 35 U.S.C. § 103

The Examiner rejects claims 1-5 and 7-8 under 35 U.S.C. § 103 as being unpatentable for obviousness over Higuchi, et al. (1992), in view of either Gershoni, et al. or Krause, et al. Higuchi, et al. (1992) contains essentially the same disclosure as Higuchi, et al. (1993). Applicants therefore traverse the Examiner's rejection on the same grounds as is specified in Section III of this Amendment with regard to the Examiner's rejection of original claims 1-5 and 7-8 for obviousness based on the combination of Burg, et al. or Higuchi, et al. (1993), in view of either Gershoni, et al. or Krause, et al.

V. Rejection Of Claim 6 Under 35 U.S.C. § 103

The Examiner rejects claim 6 under 35 U.S.C. § 103 as being unpatentable for obviousness over Burg, et al., Higuchi, et al. (1992) or Higuchi, et al. (1993), in view of either Gershoni, et al. or Krause, et al., and in further view of Renzoni, et al. Original claim 6 corresponds to new claims 37 and 38. Applicants traverse the Examiner's rejection on the

same grounds as is specified in Section III of this Amendment since the Examiner's rejection is based on an improper combination of references.

VI. Rejection Of Claims 1-2 And 9-11 Under 35 U.S.C. § 103

The Examiner rejects claims 1-2 and 9-11 under 35 U.S.C. § 103 as being unpatentable for obviousness over Lee, et al. in view of any one of Burg, et al., Higuchi, et al. (1992) or Higuchi, et al. (1993). Applicants traverse the Examiner's rejection on the grounds that Lee, et al. is not prior art relative to this application since Lee, et al. is not a paper published by another under 35 U.S.C. § 102(a).

Lee, et al. lists Linda G. Lee, Charles R. Connell and Will Bloch as co-authors. Linda G. Lee and Charles R. Connell are currently named as co-inventors of this application. Lee, et al. is only a prior art reference if Will Bloch is "another" under 35 U.S.C. § 102(a) due to his contribution to inventive subject matter disclosed in Lee, et al.

Applicants submit herewith an In re Katz type Declaration by Will Bloch in which he states that did not contribute to inventive subject matter disclosed in Lee, et al. In view of the Declaration by Will Bloch, Lee, et al. is not a prior art reference since Lee, et al. is not a paper published by another under 35 U.S.C. § 102(a). Without Lee, et al. as a supporting reference, the Examiner's present rejection is clearly unsupported and should be withdrawn.

VII. Rejection Of Claim 12 Under 35 U.S.C. § 103

The Examiner rejects claim 12 under 35 U.S.C. § 103 as being unpatentable for obviousness over Lee, et al. in view of any one of Burg, et al., Higuchi, et al. (1992) or Higuchi, et al. (1993) and in further view of Renzoni, et al. Applicants traverse the Examiner's rejection on the grounds that Lee, is not prior art as specified in Section VI of this Amendment and on the grounds that the Examiner's rejection is based on an improper combination of references as specified in Section VI of this Amendment.

CONCLUSION

In light of the Amendments and the arguments set forth above, Applicants earnestly believe that they are entitled to a letters patent, and respectfully solicit Examiner to expedite prosecution of this patent application to issuance. Should Examiner have any questions, Examiner is encouraged to telephone the undersigned.

Respectfully submitted,

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